Molecular Biology

IN VIVO STRATAGIES TO IDENTIFY DNA-BINDING SITES OF *PSEUDOMONAS AERUGINOSA* ALGZ

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Pseudomonas aeruginosa is a major respiratory tract pathogen in patients with the genetic disease cystic fibrosis (CF). Emergence of *P. aeruginosa* strains producing the exopolysaccharide alginate is correlated with a severe disease outcome. AlgZ, a ribbon-helix-helix DNA-binding protein, controls alginate production by binding to and activating *algD*, the first gene of the alginate biosynthetic operon. In order to identify other *P. aeruginosa* genes directly regulated by AlgZ at a genome wide level, an *in vivo* crosslinking and immunoprecipitation (IP) assay was developed. Cultures of the mucoid CF isolate FRD1 were grown, and wild-type AlgZ or AlgZK18 was crosslinked via formaldehyde to DNA targets. FRD2234, which expresses AlgZK18A, lacks *in vitro* DNA binding activity. Specific antibody generated against AlgZ was used to immunoprecipitate the AlgZ-DNA complexes from the total genomic DNA. Reverse crosslinking was then employed to remove bound DNA from the DNA-protein complexes. FRD2234 was used to distinguish between nonspecific and specific DNA binding of AlgZ. A second method of *in vivo* genomic binding site analysis used the crosslinking method utilized above and a Ni-NTA magnetic agarose bead column to isolate DNA targets of 6x His-tagged AlgZ. In this system, an arabinose inducible 6x His-tagged AlgZ (in strain FRD2231), or AlgZK18A (in strain FRD2600) constructs were used to define AlgZ DNA binding targets within the cell. The obtained DNA fragments were used in PCR with primers from known AlgZ targets. This yielded positive results indicating that the technique is effective. These DNA fragments will then be cloned and sequenced in order to identify genes that are regulated by AlgZ in the *P. aeruginosa* genome. Further studies will include assays to confirm AlgZ regulation of these identified genes.